STAIN TECHNOLOGY

VOLUME 21 JANUARY, 1946 NUMBER 1

A MYELIN STAINING METHOD FOR OLD MATERIAL

C. A. ERSKINE, *Department of Anatomy, King's College, Newcastle upon Tyne, England*

It is sometimes necessary to study old formalin-fixed specimens of the central nervous system, or more rarely of formalin-fixed material which has been preserved in glycerin or alcohol. In these instances it may be desired to make use of a myelin stain. Specimens of this nature very often give unsatisfactory results with the usual myelin methods because the fresh material has not been fixed in the manner required by the majority of such methods.

The following method has been found useful in such cases and might also be applied to those samples of neural tissue which are found to be refractory to the usual myelin stains; it is also suitable for use with paraffin embedded tissue. A further advantage, when it is used with routine material, is rapidity, since the mordant is not applied to the specimen in bulk, and when applied to the individual section the time required may be reduced to one or two hours. It has been used in a formalin-fixed human brain stem which had been mounted in glycerin for four years as a museum specimen, and also in old 10% formalin-fixed specimens, types which gave poor results by a variety of other myelin methods.

The mordant used is iron alum which is followed by a hematoxylin stain in accordance with the usual practice of myelin staining. In the method here described the stain is used in a dilute medium of hot Li_2CO_3, intensified in cold Li_2CO_3 solution, and the differentiation is completed by the use of a mixture of dilute $KMnO_4$ and alcohol.

METHOD

The hematoxylin stain is made up of one part of 10% ripe hematoxylin and nine parts of a solution of 5 ml. of saturated aqueous Li_2CO_3 in 95 ml. distilled water. This stain must be used at once. The block, after preliminary washing and dehydration with dioxane, is embedded in paraffin, cut at 20 to 25 μ and mounted in glycerin-albumin. Then it is treated as follows:

1. Bring the sections down to water and mordant overnight in 4% iron alum (Heidenhain).

2. Following the mordant, wash the section quickly in distilled water.

3. Place the slide on a hot plate at 50° C. and cover with a few drops of the stain for 5 minutes.

4. Flood the section with fresh stain and leave for 10 to 15 minutes. It may be noted that the heating of the section, apart from the known acceleration in staining, brings about a fairly satisfactory differentiation.

5. Remove the slide from the hot plate, drain, and wash off the excess stain completely with the dilute Li_2CO_3 solution.

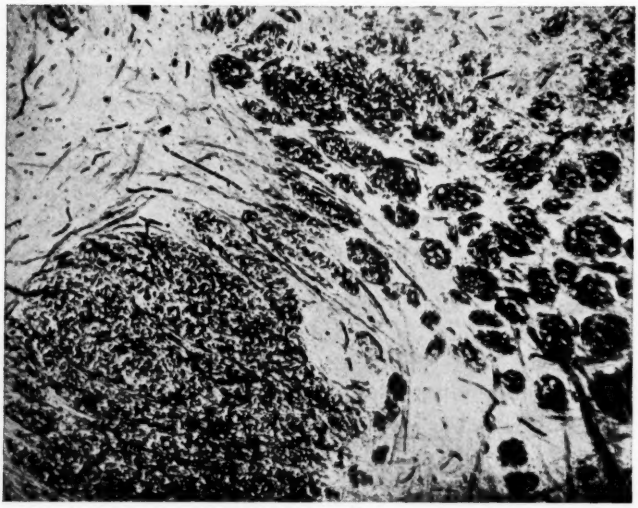


Fig. 1. Fine fiber tracts at the lateral side of the floor of the IV ventricle (fasciculus solitarius and area fasciculata). Human brain stem from museum specimen four years old. × 70.

6. Wash out the Li_2CO_3 with distilled water.

7. Flood the section with 90% alcohol for 2 to 3 minutes.

8. Take one part of a 0.25% solution of $KMnO_4$ in distilled water and add it to nine parts of absolute alcohol; a few drops of this mixture are at once placed on the slide and renewed two or three times until the gray matter can be seen to assume a light yellow color. This should take 1 to 3 minutes.

9. The section is then washed in 90% alcohol, dehydrated in absolute alcohol, cleared in xylene and mounted in balsam.

The myelin fibers appear bluish black against a pale yellow background and the transparent cell masses are lightly toned or almost colorless. If the differentiation is carried out carefully, the finer fibers show up in good contrast, as may be seen from the accompanying photomicrograph of a section through the fasciculus solitarius and neighboring structures. The best results are obtained with brain stem and spinal cord. Some thick sections tend to become detached, due it appears to the action of the Li_2CO_3 rather than to the warming of the slide; since the staining is carried out on the slide, the sections can be manipulated without much difficulty.

My thanks are due to Mr. Albert Young for the photomicrograph.

HISTOCHEMICAL TESTS FOR PROTEINS AND AMINO ACIDS; THE CHARACTERIZATION OF BASIC PROTEINS

J. A. SERRA, *Centro de Estudos Biológicos, Faculdade de Ciências, Universidade de Coimbra, Portugal*

ABSTRACT.—**For cytophysiological work it is important to have ways of demonstrating proteins and amino acids and especially of characterizing basic and non-basic proteins. The author presents a review of the more usually employed histochemical reactions for amino acids and proteic compounds in general, with several modifications which increase their sensitivity, or specificity and localization. The author describes the histochemical arginine reaction, recently introduced by him, by means of which the characterization of basic and non-basic proteins can be easily accomplished in every laboratory without costly apparatus; this reaction serves also for the demonstration of proteins in general. The application of protein histochemical tests for quantitative purposes is discussed in connection with the characterization of the basic proteins and the determination of the relative concentration and the active metabolic changes of proteic compounds.**

INTRODUCTION

The histochemical characterization of proteins and their components has recently acquired a renewed importance in connection with cytophysiological work on nucleoproteins and other cell components (see, for instance, Brachet, 1940; Caspersson, 1940, 1941; Hydén, 1943; Serra and Queiroz Lopes, 1944, 1945; and the bibliographies in Schultz, 1943 and Greenstein, 1944). For these studies it has become of importance not only to demonstrate the proteic nature of a certain cell structure, but also to distinguish between different protein classes and especially between basic and non-basic proteins.

One of the means of accomplishing this characterization is ultraviolet microspectroscopy, developed by Caspersson and co-workers (detailed descriptions in Caspersson, 1939, 1940). A strong absorption at 2600 Å indicates the presence of purine and pyrimidine compounds, which generally belong to nucleotides, of the pentose (ribonucleic) or the desoxypentose (thymonucleic) types. Owing to the presence of tyrosine, tryptophane and phenylalanine, the protein component of the nucleoproteins gives an absorption at about 2750–2900 Å; according to Caspersson, a shift of the absorption maximum

in this region to the longer wavelengths signifies that the protein is rich in hexone bases, which is characteristic of the basic or histone type proteins. If such a shift does not occur, the protein is considered to be of a non-basic or 'superior' type.

However, the demonstration of the basic type of the nucleoproteins by this method has been doubted, because histones can give an absorption at 2800 Å, the lower wavelengths of the absorption region (Mirsky and Polister, 1943; cited by Schultz, 1943). It is therefore of interest to have another independent means of identifying the basic proteins. We present here a description of such a method, which is practicable in any laboratory without costly apparatus; at the same time we make, so far as is possible for us, an up-to-date critical review of the histochemical tests for proteins and amino acids.

Preparation of the Materials

The materials may be sectioned by the freezing microtome, the reactions being executed without fixation, but in general for histochemical work it is better to fix the pieces in convenient fixatives. For all histochemical tests described below, except the reaction for SH groups, we have used with good results a fixative composed of 2 vol. of 96% alcohol, 1 vol. of commercial formalin (40% formaldehyde) and some drops of glacial acetic acid in 10 ml. of the mixture. Afterwards the pieces are well washed with running, and finally with distilled, water.

Of course, other cytological fixatives may be also employed but it is necessary to avoid colorations or precipitates caused in the tissues by the fixatives. Moreover, the materials may be embedded in paraffin and sectioned in the usual manner or stored for some time in 70% alcohol, but in these cases it is better to harden the tissues again before the reactions are executed.

This hardening, which is also necessary when fixatives without formaldehyde have been used, is done by a treatment with 10% formaldehyde (1 vol. of commercial formalin to 3 vol. of water) for 12–24 hours, followed by a thorough washing. Other references to the preparation of the materials will be made when dealing with each individual reaction.

Tests for Amino Acids and the Lower Peptides

When operating on fixed and washed material, in general it is not expected to find free amino acids, owing to the fact that these are relatively soluble. The majority of these compounds dissolve in water, though some of them, like aspartic acid, cystine, leucine,

methionine, phenylalanine, tyrosine and tryptophane are difficult to dissolve in cold water. Only in certain materials, such as plant sproutings and cotyledons, there is a relatively great amount of free amino acids; on the contrary, what is generally of interest is the characterization of amino acids bound in proteins.

In this section descriptions are given of the two histochemical reactions more commonly used to-day for the characterization of free or bound amino acids in general, and another test especially employed for sulfhydryl groups. Other reactions specific for certain amino acids will be dealt with under "Tests for proteins".

1. *The ninhydrin reaction* (Klein, 1928; Winterstein, 1933; Lison, 1936; Lloyd and Shore, 1938; Ries, 1938). Procedure of Serra and Queiroz Lopes, 1945: The fresh materials or the fixed pieces or sections are immersed in a mixture of equal volumes of (a) a 0.4% solution of triketo-hydrinden-hydrate (ninhydrin) in distilled water and (b) a phosphate buffer of pH 6.98. The reaction vessel, for instance a small watch glass covered with a glass plate, is now placed on a boiling water bath, where it is allowed to stand in the vapor for 1–2 minutes after it has reached boiling-point. A blue or violet coloration, which develops while on the bath or after cooling, indicates the presence of amino acids, free or bound in peptides or proteins.

For microscopical observation the sections or pieces are mounted directly in pure glycerin, and the tissues squeezed, if necessary. The preparations, which can be cemented with a cement made by adding 80 g. of collophonium to 20 g. of carefully heated lanolin (Romeis, 1932) must be observed in the same day, since the coloration rapidly fades away.

The ninhydrin solution must be freshly prepared, as it is unstable, and the phosphate buffer must not be too concentrated; the mixture of 6 ml. of a *M*/15 solution of secondary sodium phosphate (11.1876 g. $Na_2HPO_4.2H_2O$ per liter) and 4 ml. of *M*/15 primary potassium phosphate(9.078 g. $K\ H_2PO_4$ per liter) gives a pH of 6.98 and has always given us good results.

With regard to *specificity*, it is known that the reaction is given by all amino acids except proline and hydroxyproline, by peptides, proteins, and also by a series of other compounds like amines, aldehydes, sugars with free aldehyde or keto groups and by ammonia and ammonium salts. However, with compounds other than amino acids and proteides, the reaction is much less sensitive and sometimes it gives a more reddish color. In general it is easy to exclude the possibility of these compounds being present, by their solubility and localization. It must also be remembered that the intensity of the

ninhydrin reaction varies according to the nature of the amino acid and the binding of this in the peptides.

The coloring formed during the reaction can diffuse and be *absorbed* by several cell structures. This commonly happens when the heating is exaggerated and when compounds easily soluble are present, for instance after a weak fixation. It is, therefore, recommended to employ fixatives which harden the tissues, as we have said above. To be sure that a secondary impregnation or adsorption of the coloring has not taken place, the following test may be executed: A small weight (some milligrams) of a pure amino acid, such as glycine, is dissolved in distilled water; an equal volume of phosphate buffer of pH 6.98 and a few drops of 0.4% ninhydrin solution are added; it is boiled slowly and cooled for 20–30 minutes. The ninhydrin employed must be completely consumed — by addition of more amino acid solution. The colored liquid of this reaction is now used to immerse the pieces, with boiling, etc., as for a ninhydrin reaction. If then a certain structure shows a coloration, this means that an absorption or adsorption has taken place and a positive ninhydrin reaction in the same structure does not necessarily demonstrate a proteic or amino acid nature.

2. *The alloxan reaction* (Winterstein, 1933; Lison, 1936; Ries, 1938). An alcoholic 1% solution of alloxan gives with amino acids and proteic compounds a pink coloration, after a long time at room temperature, or rapidly if the reaction is activated by heating in a boiling water bath. In our experience, this test is relatively insensitive; besides this, the coloring formed diffuses easily, so that the reaction can be indistinctly localized. With fixed materials the reaction is weak.

The test must be carried out in neutral solutions; this is attained by addition of a phosphate buffer, as described for the ninhydrin. This reaction is not specific for amino acids and proteins, as it is also given by compounds with free NH_2 and perhaps SH groups (see Winterstein, 1933).

3. *The reaction for sulfhydryl groups* (Lison, 1936; Ries, 1938; Serra and Queiroz Lopes, 1945). This reaction has been extensively used for the study of the distribution of the tripetide glutathione. One of the better methods of accomplishing the reaction is that of Giroud and Bulliard (see Lison, 1936), which gives a stable red coloration, while other methods produce a violet color rapidly fading away.

The pieces are immersed for some seconds (in general an excess of time does no harm) in a 5% aqueous solution of zinc acetate. Directly afterwards they are treated with a 10% aqueous solution of sodium

nitroprusside, containing about 2% concentrated ammonia. The pieces acquire a bright red coloration, which attains its maximum in 3–5 minutes. Afterwards they are mounted in pure glycerin for microscopic observation, if necessary with a preliminary washing in distilled water.

The materials may be studied freshly or after fixation. It must be noted, however, that the majority of the fixatives hinder the reaction. We obtained good results with a fixation in 10% neutral formaldehyde during 2–15 hours at room temperature. A more prolonged action of this fixative also hinders the reaction; it is recommended, therefore, that if possible 2–4 hours of fixation be used.

The results of the reaction have different meanings according to the fixation, washings, etc., because the glutathione is partly soluble. When the tissues are treated several times with a 10% solution of trichloroacetic acid for 15 minutes, the glutathione is dissolved and only "fixed", that is, proteic sulfhydryl groups remain in the preparation. It is still possible not only to demonstrate the existing SH groups but also to reduce SS groups to SH groups, by means of a pretreatment of the materials with a solution of 10% KCN for 10 minutes in a small stoppered bottle (the cyanide solution can be weakly alkalinized with potassium hydroxide, to make its use safe).

The reaction has been recognized as well localized, but in case of doubt a test of secondary impregnation can be made in the way described above for the ninhydrin.

Tests for Proteins

Under this heading will be described tests which are also given by free amino acids, but whose characterization is principally important when they are bound in proteins.

1. *The biuret reaction* (Klein, 1928; Winterstein, 1933; Lloyd and Shore, 1938; Calvery, 1938). The pieces are immersed in strong NaOH or KOH solution in a watch glass and some drops of a 1% aqueous solution of $CuSO_4$ are then added with stirring. A blue-violet coloration indicates the presence of peptides or proteins.

The reaction is given by the peptide linkage when the peptides are composed of at least three amino acids. The color is more reddish with the simpler peptides. For cytological or histological work, the reaction has the disadvantage of requiring a strong alkaline reaction, which tends to dissolve the protoplasm. To avoid a serious dissolution the tissues must be hardened, for instance with formalin (10% formaldehyde during 24 hours, followed by a thorough washing). This reaction has also the disadvantage of being insensitive.

2. *The xanthoproteic reaction* (Klein, 1928; Winterstein, 1933;

Lison, 1936; Calvery, 1938; Lloyd and Shore, 1938; Ries, 1938). The pieces are treated for some minutes with concentrated HNO_3 until they become intensely yellow. After a washing in distilled water, immerse in a diluted ammonia solution, or expose the pieces to ammonia vapors. The color changes to orange. The observation can be made by mounting directly in pure glycerin.

The reaction is due to the presence of tyrosine, phenylalanine or tryptophane in the protein molecule, and is also given by all phenolic compounds. Among the peptides, only the protamines do not show a positive reaction. To withstand the treatments, a strong fixation is recommended, though the reaction can also be performed on fresh materials.

3. *The arginine reation* (Serra, 1944). This is the application to histochemistry of the arginine reaction used in microchemistry. The procedure of Serra (1944b) is the following:

(a) Before the reaction the pieces or sections are hardened with 10% formaldehyde during 12–24 hours, the formalin being afterwards well washed out. (If the fixative contains formalin this step can be omitted).

(b) Immerse the pieces for 15 minutes in a mixture consisting of 0.5 ml. of diluted α-naphthol; 0.5 ml. of *N* NaOH; and 0.2 ml. of 40% aqueous urea solution. The diluted α-naphthol is prepared at the moment of use by diluting a stock solution (1% crystallized α-naphthol in 96% alcohol) 1:10 with 40% alcohol. The watch glass containing the liquids is placed in an ice-bath and the temperature of the reaction fluid inside it must be 0-5° C.

(c) After 12–15 minutes add 0.2 ml. of a 2% solution of NaOBr. This reagent is allowed to act for 3 minutes and the solution must be well stirred during this time. The 2% NaOBr must be freshly prepared by pouring 2 g. (or approximately 0.7 ml.) of liquid bromine into 100 ml. of 5% NaOH, with agitation and cooling.

(d) Add another 0.2 ml. of 40% urea solution, stir, and immediately afterward,

(e) Add another 0.2 ml. of 2% NaOBr and stir well. The coloration attains its maximum after 3–5 minutes and would last only for a short time if it were not stabilized. To stabilize the coloration:

(f) Take the pieces out of the reaction mixture and immerse in pure glycerin for 2–3 minutes and then transfer to fresh glycerin. Repeat the operation another two or three times. The passage through 4 glycerin baths is sufficient to stabilize the coloration for some months, even if the pieces are left at room temperature. (We have not mentioned this improvement in any previous publication).

Besides this procedure, which we may call the normal method,

there is also another method which results in *stronger colorations* and very satisfactory preparations. To accomplish this, after step *e* the pieces are taken off the reaction liquid and immersed in NaOBr solution for not more than 3 minutes. Afterwards the coloration is stabilized in glycerin, as in the normal procedure. The pieces are mounted and observed in pure glycerin. (See Fig. 1–3.)

This reaction is specific for guanidine derivatives in which only one H-atom of one amino group is substituted by a radical of the alkyl or fatty acid type. In proteic compounds it is specific for arginine. As all proteins hitherto analyzed possess arginine in their molecules, the reaction may be used to demonstrate the presence of proteins in general, other compounds with a reactive guanidine group being rare. The test may also be used to characterize the basic proteins (see below).

4. *The tyrosine reaction (Millon's modified).* (Klein, 1928; Winterstein, 1933; Lison, 1936; Calvery, 1938; Lloyd and Shore, 1938; Ries, 1938). The procedure of Serra and Queiroz Lopes, 1945, which gives better results than the Millon's reaction, is as follows:

(a) Immerse the objects for 30 minutes in a few milliliters of the mercuric solution (composition: $MgSO_4$, 7.5 g.; $MgCl_2$, 5.5 g.; Na_2SO_4, 7.0 g.;—dissolved in 85 ml. of distilled water to which 12.5 g. of concentrated H_2SO_4 is added; after dissolving diluted to 100 ml. with distilled water). Perform the treatment in a small glass stoppered bottle, placed in a water bath which is maintained at 60° C.

(b) After the 30-minute treatment, cool the bottle in running water and allow to stand at room temperature for 10 minutes.

(c) Dilute the mercuric solution in the bottle, by addition of an equal volume of distilled water.

(d) Develop the color, adding now some drops of a freshly-prepared 1 *M* solution of sodium nitrite (6.9 g. $NaNO_2$ in 100 ml. of water).

The coloration attains its maximum in 3 minutes and lasts for some months, though it fades gradually with time. The materials are mounted and observed in pure glycerin, where they can be squeezed or squashed, if necessary. (See Fig. 4-6.)

The reaction is principally due to the presence of tyrosine in the protein molecule, and is also produced by other phenolic compounds. The method here described gives with tryptophane only a transient coloration, which lasts no more than a few minutes; it is hoped, therefore, that by this procedure this histochemical test reveals only the tyrosine in the proteins.

5. *The tryptophane reaction* (Klein, 1928; Winterstein, 1933). Reaction of Voisenet-Fürth, according to the procedure of Serra and Queiroz Lopes, 1945a:

FIG. 1. Testis of a male frog. Fixation: alcohol-formol-acetic acid. Arginine reaction. Mounted in glycerin and squashed. At the lower right a group of spermatozoa heads, at the left several spermatocytes in I division of the meiosis. Photographed with a green filter. ×465.

FIG. 2. A group of spermatozoa of the snail *Helix aspersa* Müll in an advanced stage of the spermiogenesis. Fixation: alcohol-formol-acetic acid. Arginine reaction. Mounted in glycerin. At the top their heads, below them the tail in formation and the remnants of the granulous cytoplasm. ×465.

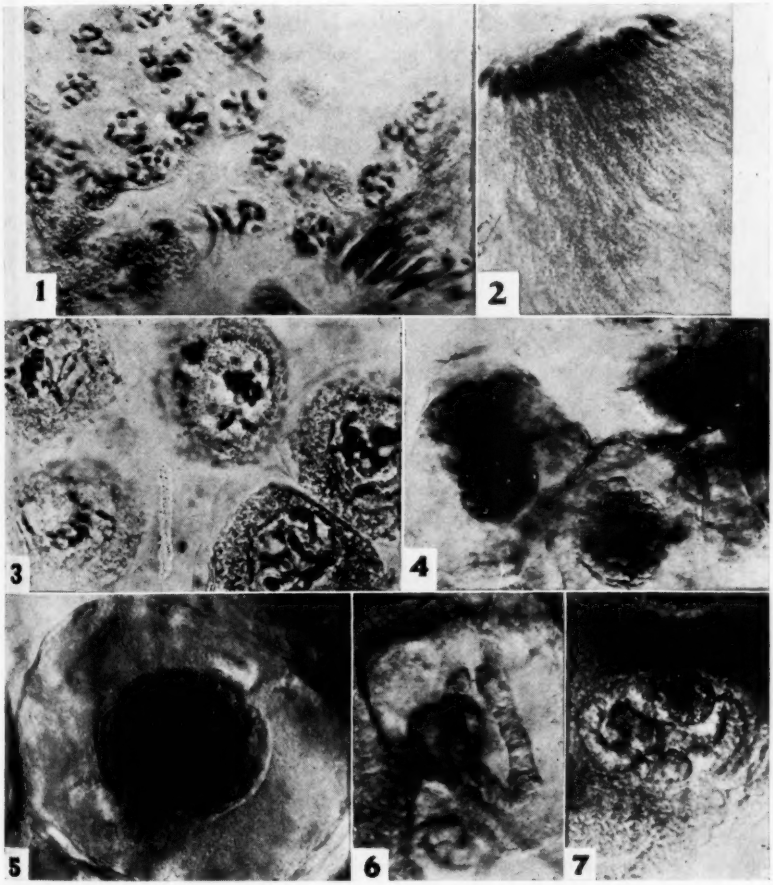
FIG. 3. Pollen mother cells of *Vicia faba* L, fixed as for Fig. 1 and 2. Arginine reaction. Squashed in glycerin. The meiotic chromosomes give a strong reaction, the cytoplasm is also relatively rich in basic proteins. ×750.

FIG. 4. Ovary of a female frog. Fixation alcohol-formol-acetic acid. Tyrosine reaction. Mounted in glycerin. There is a great difference in the reaction given by the cytoplasm and the nucleus (while the arginine reaction is almost as strong in both, which makes difficult to photograph the ovules after an arginine coloration). Photographed without filter. ×350.

FIG. 5. An ovule of the same frog as in Fig. 4. Tyrosine reaction; mounted in glycerin. In the nucleus a strong reaction is given by the small and numerous nucleoli, the chromosomes and perhaps also by the nucleoplasm. Photographed without filter. ×465.

FIG. 6. A nucleus of the salivary glands of a *Chironomidae* larva Fixation as for Fig. 4 and 5. Tyrosine reaction. Only a chromosome and the nucleolus are seen, the other three chromosomes are on other planes. Photographed without filter. ×580.

FIG. 7. A cell of the salivary glands of a *Chironomidae* larva. Fixation: alcohol-formol-acetic acid. Mounted in glycerin. Several chromosomes and the great nucleolus are seen within the nucleus. The light violet coloration is very difficult to photograph, though it can be easily observed at the microscope. Photographed with a yellow filter. ×465.


1
2
3
4
5
6
7

(a) Harden the fixed pieces in 10% formaldehyde for at least 1–5 hours (an unnecessary step if a fixative with formalin has been employed); then wash well.

(b) Immerse for 3–5 seconds in an aqueous solution of sodium silicate (d = 1.1). When the materials are sufficiently hardened this step may also be omitted; it is recommended, however, that the coloration should be tried both with and without it.

(c) Immediately afterwards, immerse the pieces in the Voisenet reagent for 10–15 minutes, in a small glass stoppered bottle. This reagent is composed of 10 ml. concentrated HCl to which is added, with a thorough stirring, one drop of 2% aqueous formol and one drop of 0.5% aqueous $NaNO_2$. The reagent is prepared freshly every day and the nitrite solution must also be freshly made.

(d) Mount directly in glycerin and observe, with squeezing, if necessary. As the coloration fades, it is necessary to observe the preparations on the same day.

The reaction is given by indolic compounds, and in proteins it is specific for tryptophane, which reacts even when bound. The localization of the reaction seems to be satisfactory and the *sensitivity* is sufficient for it to be used in cytophysiological work. (See Fig. 7.)

5. *The diazo reaction* (Klein, 1928; Winterstein, 1933; Lison, 1936). According to Serra, 1945, modified: Treat the pieces for 2–3 minutes with a saturated aqueous solution of sodium carbonate; afterwards add some drops of the diazo reagent and stir the liquid well. Observe in glycerin. (The coloration develops rapidly and lasts for some days.) Preparation of the diazo-reagent: into a 50 ml. flask immersed in an ice bath, pour 1.5 ml. of a sulphanilic acid solution (dissolve 0.9 g. of pure sulphanilic acid in 9 ml. of concentrated HCl and add water to 100 ml.); add 1.5 ml. of a 5% aqueous solution of $NaNO_2$, shaking the flask meanwhile. After 5 minutes in the ice bath add, also while shaking, another 6 ml. of nitrite. After 5 minutes fill up to 50 ml. with cooled distilled water. The reagent must be prepared every day and kept in the ice chest.

The reaction gives an orange or yellow color with the histidine and the tyrosine of the proteins. According to Klein, 1928 (see also Romeis, 1932) it would be possible to distinguish between histidine and tyrosine if a xanthoproteic reaction were previously performed upon the same pieces, followed by the diazo reaction. In these conditions only the histidine would show a red color, but the two processes seriously damage the structures, so much that the coloration may finally not be distinctly localized.

Lison (1936) performs the reaction in a slightly different way and

claims that it demonstrates the presence of phenolic compounds, which would give an orange, red or blue coloration, a yellow color being only due to azoproteides without special significance.

The Characterization of Basic and Non-Basic Proteins

The arginine reaction is suitable not only for the characterization of proteins in general, but also for giving an idea of the relative richness of the proteins in this amino acid. As the coloration is more or less proportional to the arginine present (Serra, 1944b) a very intense reaction demonstrates the existence of proteins, such as the histones, with a basic character due to the excess of basic amino acids and especially of arginine. Besides the histones, only the protamines and the edestins possess a great amount of this amino acid; but as the edestins are plant globulins found only in seeds and the protamines have been encountered chiefly in fish sperms, in general the presence of a high concentration of arginine can be interpreted as indicating that the proteins are of a basic type resembling the histones.

On the other hand, a weak arginine reaction may be taken as indicating the absence of basic proteins, or at least that these can be present only in small amounts. In this case, the proteins are of the non-basic type.

Within the non-basic proteins, it is yet possible to distinguish several protein classes by their solubility. Thus, the albumins are soluble in water, while the globulins dissolve only in diluted salt, acid and alkaline solutions, and the scleroproteins, like the keratins and collagen, require strong alkali to go into solution. Though these distinctions can be clearly seen in test tube assays, the conditions in the cell do not in general allow conclusions to be reached by this method; nevertheless, the solubility has been used in cytophysiological work (for instance by Gersch, 1940).

When interpreting histochemical reactions quantitatively it is always necessary to bear in mind that certain assumptions are implicitly made, namely (1) that the color intensity is proportional, even only roughly, to the quantity of the compound which is present in the cell formation; (2) that the density of structure, or relative concentration of substance, is not very different in the parts which are being compared, for instance in the ground cytoplasm and the nucleus; and (3) the method must not give rise to secondary color impregnations or adsorptions. Condition 3 is experimentally controllable, while questions 1 and 2 are in general more or less a matter of critical judgment. But if color intensities, which in test tube assays are known to be more or less proportional to the concentration,

are very intense in a certain cell structure, say four or five times more intense in the chromosomes than in the ground cytoplasm, we may safely conclude that the relative concentration is greater in the chromosomes, because the difference in density of structure must not be so great. It is, however, always necessary to be critical about these results. The ultraviolet microspectroscopy can also be affected by condition 2.

The color intensity may be roughly measured by comparison of the apparent coloration seen at a given microscope magnification, with color charts like those used by Levine (1940). Successive dilutions of a certain dye, whose color matches well the color seen through the microscope, are prepared and painted on small squares on white drawing paper. Alternatively, the comparison can be made by means of a visual colorimeter like the Tintometer (Salisbury, England), with logarithmically graduated glasses, the tube of the colorimeter being placed close to the eyepiece of the microscope (Serra and Queiroz Lopes, 1944a).

The Concentration of Proteins in General and the Zones of Intense Metabolism

While the arginine reaction may be used quantitatively to characterize the basic proteins, the reactions for tyrosine, for tryptophane and for the protein SH groups can serve as indications respectively of the relative richness of the cell structures in proteins in general, or of the metabolic activity of a given formation.

Tyrosine seems to be present in almost all natural proteins in amounts which are not very different for the various classes, excepting principally silk fibroin, pepsin and insulin (see the tables of Calvery, 1938; and Lloyd and Shore, 1938). We can say that in the great majority of cases a strong tyrosine reaction indicates a great richness of proteins in general, and if the density of structure is not very different, the intensity of the reaction can serve as a rough quantitative estimation of the proteins present in several structures.

Tryptophane is also a constituent of the great majority of the proteins, generally in small amounts between 1 and 3%. The histones cannot be distinguished by means of their content in this amino acid. A more intense reaction can have the same significance as the tyrosine reaction, or it can be taken as suggesting the existence of a zone of intense metabolism in which peptides are being synthesized or split (Serra and Queiroz Lopes, 1945).

An intense reaction for protein SH groups (that is, for sulfhydryl groups which remain after extraction of the glutathione) has also been

supposed to demonstrate the existence of active metabolic and formative changes in the proteins (Brachet, 1940). In fact, it is known that denaturation of proteins is accompanied by an increase in reactive SH groups, and this is supposed to be due to an unfolding of polypeptide chains. It is, therefore, probable that an intense reaction for protein sufhydryl groups might be taken as indicating an active protein synthesis or breakdown. As we have emphasized above, it is also necessary to be critical in this case, when using these reactions for quantitative purposes.

REFERENCES

Brachet, J. 1940. Étude histochimique des protéines au cours du développement embryonnaire des Poissons, des Amphibiens et des Oiseaux. Arch. Biol., **51**, 167–202.

Calvery, H. O. 1938. Methods of analysis and reactions of the amino acids and proteins. Schmidt, The chemistry of the amino acids and proteins, p. 183–220. C. Thomas, Baltimore, Md.

Caspersson, T. 1940. Methods for the determination of the absorption spectra of cell structures. J. Roy Micr. Soc., **40**, 8–25.

————. 1940. Die Eiweissverteilung in den Strukturen des Zellkernes. Chromosoma, **1**, 562–604.

————. 1941. Studien über den Eiweissumsatz der Zelle. Naturwiss., **29**, 33–43.

Gersch, M. 1940. Untersuchungen über die Bedeutung der Nukleolen im Zellkern. Zts. Zellforsch., **30**, 483–528.

Greenstein, J. P. 1944. Nucleoproteins. Advances in protein chemistry. Vol. **1**, 210–287. Academic Press. New York.

Hyden, H. 1943. Protein metabolism in the nerve cell during growth and function. Acta Physiol. Scandin., **6**, Suppl. 17.

Klein, G. 1928. Allgemeine und spezielle Methodik der Histochemie. Peterfi, Methodik der wiss. Biologie, **1**, 1016–1084. J. Springer, Berlin.

Levine, N. D. 1940. The determination of apparent isoelectric points of cell structures by staining at controlled reactions. Stain Techn., **15**, 91–112.

Lison, L. 1936. Histochimie animale. Méthodes et problèmes. Gauthier-Villars, Paris.

Lloyd, D. J., and Shore, A. 1938. Chemistry of the proteins. Churchill, London.

Romeis, B. 1932. Taschenbuch der mikroskopischen Technik. R. Oldenburg, Berlin.

Schultz, J. 1941. The evidence of the nucleoproteic nature of the gene. Cold Spring Symp. Quant. Biol., **9**, 55–65.

————. 1943. Physiological aspects of genetics. Ann Rev. Physiol., **5**, 35–62.

Serra, J. A. 1942. Relations entre la chimie et la morphologie nucléaire. Bol. Soc. Brot., 2s., **16**, 83–135.

————. 1944. Eine neue histochemische Reaktion, die Reaktion des Arginins. Naturwiss., **32**, 46.

————. 1944 a. Une réaction nouvelle pour l'Histochimie: la réaction pour l'arginine. Zts. wiss. Mikr., **60**.

————. 1944 b. Improvements in the histochemical arginine reaction and the interpretation of this reaction. Portugaliae Acta Biologica, **1**, 1–7.

SERRA, J. A., 1945. Fenogénese e composição das melaninas de Mamíferos. Rev. Esc. Farmácia. Univ. Coimbra, **4**, 188–285.

SERRA, J. A., and QUEIROZ LOPES, A. 1944. Direkter Nachweis von basischen Proteinen in den Chromosomen und im Nukleolus. Naturwiss., **32**, 47.

————. 1944 a. Direkter Nachweis von basischen Proteinen im Zellkern. Chromosoma, **2**.

————. 1945. Chemical constitution of the nucleolar inclusions in growing oocyte cells. Nature, **155**, 792.

————. 1945 a. Données pour une cytophysiologie du nucléole. I. L'activité nucléolaire pendant la croissance de l'oocyte chez des Helicidae. Port. Acta. Biol., **1**, 51–91.

WINTERSTEIN, A. 1933. Aminosäuren. Klein, Handbuch der Pflanzenanalyse. J. Springer, Wien.

A RAPID HISTOLOGICAL TECHNIC FOR STAINING LATEX IN ROOTS OF TARAXACUM KOK-SAGHYZ[1]

LUCY B. ABBE[2]

ABSTRACT.—**The freezing technic described in this paper provides permanent slides of sections of the root of *Taraxacum kok-saghyz* with latex preserved in place. The following schedule is used: (1) Prefreeze the piece to be sectioned before removal from the root. (2) Mount in ice and section with chilled microtome knife. (3) Plunge frozen sections into the combination coagulant and stain prepared from Calco oil blue N. A., acetic acid and ethyl alcohol. (4) Wash in water. Aspirate if necessary. (5) Mount on a slide using Karo.**

This technic is rapid and simple. The sections are well adapted to making counts and measurements of latex tubes since there has been a minimum of latex loss. Latex is retained in place by keeping tissues frozen until introduced into the coagulant.

INTRODUCTION

Frozen sections have been useful for a study of the latex system in roots of *Taraxacum kok-saghyz*. In this study it was desirable to obtain permanent mounts of uniformly thick sections with the latex preserved and stained in place. It was also important to find a rapid technic which could be learned quickly by unskilled workers. For this reason all unnecessary steps were avoided in favor of speed and ease of handling.

PROCEDURE

1. *Freezing* — To prevent loss of latex the part of the root to be sectioned is frozen quickly and is kept frozen until the sections are allowed to thaw in the combination stain and coagulant. From the

[1]Cooperative investigations by the Rubber Investigations, Bureau of Plant Industry, United States Department of Agriculture, the Botany Department and the Division of Agronomy and Plant Genetics of the University of Minnesota.

[2]Agent (Associate Anatomist), Rubber Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

The writer is grateful to Miss Olga Hansen of the Botany Department for valuable suggestions concerning the technic; to Dr. E. C. Abbe of the Botany Department, Dr. Hayes of the Division of Agronomy and Plant Genetics and Dr. W. G. Whaley of the Division of Rubber Plant Investigations, for critical reading of the manuscript; and to R. W. Henderson of the Division of Rubber Investigations, under whose direction the work was done, for making the rubber determinations and calculating the correlation co-efficient.

whole root or a piece of root at least 4 or 5 cm. long, a segment of root about 1 cm. in length is frozen quickly with dry ice (solid CO_2) or liquid CO_2. The time required for freezing depends mainly on the diameter of the root. Using solid CO_2, 5–10 seconds was required to freeze properly a root 3 mm. in diameter.

2. *Mounting for Sectioning* — A segment of well-frozen root 5 mm. in length is then mounted on a dry ice or liquid CO_2 freezing attachment. Care should be taken not to let the root thaw during the above process, in which case it will turn brown and the latex will form adhesive strands which stick to the knife and pull out during sectioning.

3. *Sectioning* — A sliding microtome is used for sectioning. The microtome knife may be kept at freezing temperature by fastening a piece of solid CO_2 above the cutting edge. The root segment should be frozen to the point where it is firm but not brittle. If the root is frozen too hard or if the knife is too cold the sections will crack. Sections may be cut 20–100 μ thick but should be of uniform thickness for comparative studies of the latex tubes. Sections are removed from the knife with chilled forceps and plunged into the combination coagulant and stain where they are allowed to thaw. Preliminary extraction of lipids, resins and similar compounds did not seem essential.

4. *Staining* — The stain is made as follows: One-half gram of Calco oil blue N. A. is added to 100 ml. of 50% ethyl alcohol. Shake well and allow to stand 48 hours. Part of the dye will dissolve. Of the resulting clear blue solution of Calco oil blue 95 ml. are then decanted, filtered, and mixed with 5 ml. of glacial acetic acid in stock bottle 2. The primary function of the acetic acid is to help coagulate latex.

The staining solution is used only while it remains clear. If it becomes slightly cloudy, staining will be poor and the sections will be covered with dye crystals which are difficult to remove.

Staining for 45 minutes is usually sufficient to give a bright blue stain to the coagulated latex (Fig. 1). The stained sections should be rinsed quickly in distilled water before mounting.

Whittenburger (1944), Artschwager (1943) and Addicott (1944) have previously discussed the use of Calco oil blue as a latex stain.

5. *Mounting*—Sections are mounted in a drop of a solution of 0.1% carbolic acid, 66⅔% high grade white Karo, and 33⅓% water. The Karo solution dries to a fine hard mount in a reasonably dry atmosphere, and has been useful when alcohols and xylenes are to be avoided during mounting. The use of Karo is previously mentioned by Johansen (1940, see p. 24), Patrick (1936), and Addicott (1944).

If trapped air bubbles are objectionable, they may be removed by aspiration while the sections are being washed.

Slides made by the above method have remained in excellent condition for almost two years.

Practical Application of the Method — Sections made by the above method have been used to determine the degree of correlation be-

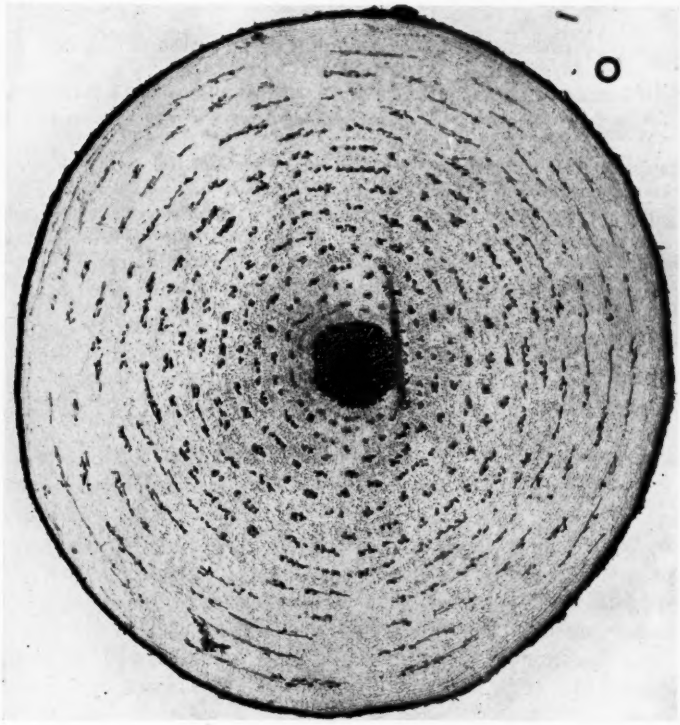


Fig. 1. Cross-section of *Taraxacum kok-saghyz* root showing darkly stained latex strands in the concentric rings of latex tubes. × 22.

tween rubber percentage and certain characteristics of the laticiferous system in the root of *Taraxacum kok-saghyz.*

That latex and latex tubes are well preserved by this method is indicated by the high degree of correlation between the per cent of rubber in the roots and visual ratings for latex density of sections taken from the same roots. Using 90 roots from as many different plants, the correlation between the latex density ratings and the

per cent of rubber in the same roots was .753. This greatly exceeds the correlation coefficient of .267 required for significance at the 1% level.

The chief advantages of the method described in this paper are (1) retention of relatively normal cell size and (2) ease and speed with which sections may be prepared.

REFERENCES

ADDICOTT, FREDERICK T. 1944. A differential stain for rubber in Guayule. Stain Techn., **19**, 99–102.

ARTSCHWAGER, ERNST, and MCGUIRE, RUTH C. 1943. Contribution to the morphology and anatomy of the Russian dandelion (*Taraxacum kok-saghyz*). U. S. D. A. Tech. Bull. **843**.

JOHANSEN, D. D. 1940. Plant Microtechnique. McGraw-Hill, New York.

PATRICK, RUTH. 1936. "Karo" as a mounting medium. Science, **83**, 85–6.

WHITTENBURGER, ROBERT T. 1944. Oil blue N. A. as a stain for rubber in sectioned or ground plant tissues. Stain Techn., **19**, 93–8.

A GOLD CHLORIDE METHOD FOR MOTOR END PLATES

WILBUR V. COLE, *Department of Anatomy, Kirksville College of Osteopathy & Surgery, Kirksville, Missouri*

Since Ranvier[1] established the method of precipitating a metallic salt in nervous tissue by means of an acid, this technic and modifications of it have been used extensively. However, in the staining of terminal axons and the motor end plates of striated muscle, the results have too often been erratic. The method herein described eliminates much of the variation due to the solutions used; it has been used with uniform results in the preparation of several thousand teased specimens.

Three solutions are used in this staining method:

(1) Citric acid in solution—10% citric acid in normal saline solution.

(2) Gold chloride,[2] 1% (1 gram gold chloride in 100 ml. distilled water). This solution should be at least 24 hours old for good results. It does not deteriorate with age. The gold chloride solution can only be used once.

(3) Aqueous formic acid 20%.

METHOD

1. Remove striated muscle and immerse in 10% citric acid. Let the tissue remain in this solution for a minimum of 10 minutes and a maximum of 30 minutes.

2. Place directly (without washing) in the 1% solution of gold chloride. Let the tissue remain in this solution for about 60 minutes, or until the color of the tissue is a dark yellow. It is not necessary to keep the solution in the dark. However, direct sunlight is detrimental to the staining process. For best results, tissue should not be greater than 4 mm. along two edges when placed in the gold chloride. If it is necessary to use larger pieces of tissue, the time in solution may be proportionally increased.

3. Without washing, place tissues in a 20% solution of formic acid. Allow the tissue to remain in this solution a minimum of 10 hours and maximum of 20 hours.

4. Place tissue without washing in a 50% mixture of 95% methyl alcohol and pure glycerin for several hours. Remove the top of the

[1]Ranvier, R. 1880. On the terminations of nerves in the epidermis. Quart. J. Micr. Sci., 80, 456.

[2]Gold Chloride, auric, Mallinckrodt Chemical Works, St. Louis, Mo.

container and when the alcohol has evaporated transfer the tissue to pure glycerin. The tissue will keep in glycerin for months without deterioration.

To mount, tease muscle fibers and place on slide in a drop of pure glycerin. Place cover glass over the teased muscle and seal with 60% Clarite solution, a synthetic resin,[3] or Harkite, a synthetic resin in toluene.[4] The evaporation of the alcohol in step 4 helps to prevent the formation of air bubbles in the finished slide. By using small pieces of tissue, artifacts, such as air bubbles and distorted muscle fibers, are prevented.

This method results in permanent sections. The muscle fibers stain red-blue while the motor end plate and medullated axon stain black (Fig. 1). The nuclei of the muscle fibers do not stain with this technic, but the striations of the muscle fibers are very well demonstrated. Under favorable circumstances this method also demonstrates myoneural bundles and neurotendonous endings.

[3]Central Scientific Co., New York, N. Y.
[4]Hartman-Leddon Co., Philadelphia, Pa.

Fig. 1. Motor end plate (rat). Modified gold chloride. × 940 and enlarged × 3

DEMONSTRATION OF THE GROUND SUBSTANCE OF CARTILAGE, BONE, AND TEETH

E. B. Ruth, *Department of Anatomy, College of Medicine, University of Cincinnati, Cincinnati, O.*

Abstract.—**A slab of bone about 5 mm. thick is decalcified in 5% HCl, washed, and placed for several days to 2–3 weeks in 3% KOH in 20% glycerin (if the bone is medium sized); for small bones the KOH should be decreased to 1%, and for large bones it may be increased to 5%. The solution is changed frequently. When the bone begins to dissociate, it should be removed and washed in water till all traces of alkali are removed. The specimen is passed through 3 changes of dioxane into paraffin, and then through a second paraffin bath into the final paraffin. Sections are cut at 10–12 μ and stained with VanGieson's picro-fuchsin or with orcein.**

Several technics for unmasking the fibrillar ground structure of the supporting tissues have been described in the literature, but the results obtained by these methods have proved disappointing. In attempting to display the fibrillar structure of bone, the method herein described has proved satisfactory in this laboratory, and has been applied to the study of the structure of teeth and cartilage also, with instructive effects.

Digestion of tissues by ferments and alkalis for the study of structural details that otherwise remain masked is a recognized technic. It has been applied in one form or another to the study of practically every tissue in the body. The following procedure depends upon KOH in aqueous solutions of different strengths to wash out the amorphous interstitial substance and unmask the fibrillar structure of the supporting tissue in question. Bone is used here, but teeth and cartilage are treated in the same manner.

A slab of bone about 5 mm. thick is decalcified in 5% HCl. Bone in any condition may be used—fresh, desiccated, fixed, or unfixed. Fresh, unfixed bone seems to respond best to this treatment. If the specimen is greasy, it may be degreased with a suitable fat solvent (acetone, ether, benzene, or gasoline are all satisfactory) before decalcification.

Decalcification is followed by washing to remove the acid, and the slab then is treated with a KOH solution made as follows (Mall, 1906):

Water	77 ml.
Glycerin	20 ml.
KOH	3 g.

This treatment is continued until signs of dissociation appear, and may require from several days to two or three weeks, according to the structural nature of the ossein in a given species. Experience will show that this varies in different animals, and a single rule cannot be formulated to cover all cases. The solution should be changed as often as it begins to appear cloudy. Signs of dissociation are recognized by a fuzzy appearance of the edges of the tissue, or by the inception of actual fragmentation. When this begins, the specimen should be removed immediately to water, and should be washed until all traces of the alkali are removed—preferably in running water overnight. In some cases a certain amount of disintegration of the tissue is necessary before the proper amount of the amorphous interstitial substance has been removed and the fibrillar structure becomes clearly apparent. Again experience must dictate the extent to which this should be carried.

The discussion this far applies to slabs of medium-sized bones; i. e., bones of the size of the femur of the rat to those of the size of a dog femur. Smaller bones, and delicate bones with a very thin cortex are treated with the same reagent, but with the KOH reduced to make a 1% solution. If such bone shows no change after a week of this treatment, it may then be transferred to the more concentrated potash solution. Large bones (i. e., femora of human adults and larger) may need more vigorous treatment. After an initial period in the 3% potash solution these specimens may be transferred to a 5% solution, if they have not shown indications of dissociation. In the stronger concentration the tissue must be watched carefully, as changes may occur rapidly and destroy the ossein completely.

Thorough washing of the macerated slab is followed by embedding in paraffin for sectioning. The specimen is passed from water into pure dioxane, using three changes, with one hour in each, for medium-sized and small bones. Slabs from large bones may be left in dioxane for a longer time for complete dehydration, without danger of too much hardening. From dioxane the bone is passed into melted paraffin—one hour in each of three paraffin baths, or until all dioxane has been removed. It is well to stir the paraffin occasionally, as the dioxane does not diffuse readily.

Sections cut at 10μ to 12μ are satisfactory. They may be stained with Van Gieson's picro-fuchsin, or with orcein. The synthetic orcein produced by MacAndrews and Forbes Company, Camden, N. J.[1], gives the best results. The bones of animals having Haversian systems show an unusual effect in that the fibrillae of the alter-

[1]Now sold by Eastman Kodak Co., Rochester, N. Y.

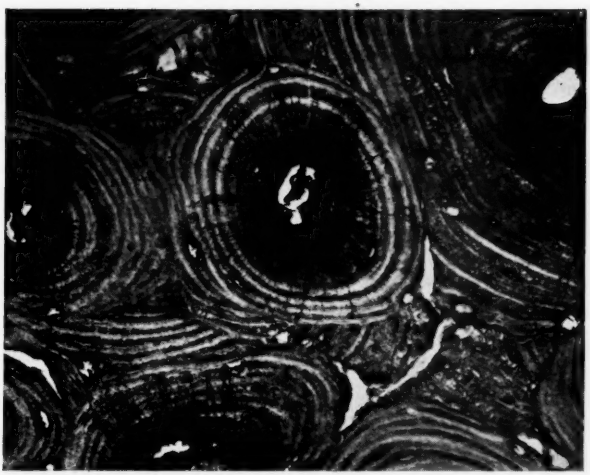


Fig. 1

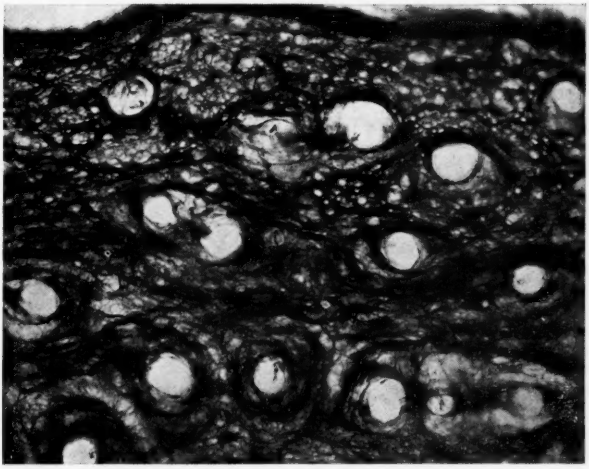


Fig. 2

Fig. 1. Section of a Haversian system of a human femur, stained with orcein. This type of bone shows a regularity in orientation of fibrillae, and the diffuse lamellae are stained selectively with orcein. With picro-fuchsin the compact lamellae are stained with fuchsin. Mag. 90×.

Fig. 2. Section through the cortex of the femur of a pigeon. Fibrillar structure is less regularly organized, and does not stain differentially. Dissociation has been carried considerably farther than in Fig. 1. Mag. 200 ×.

nating compact and diffuse lamellae stain differentially with these stains (Fig. 1), as described elsewhere (Ruth, 1945), when the proper degree of dissociation has been effected. Bones of animals in which the fibrillar structure is organized less regularly (Fig. 2) do not exhibit this differential staining reaction. The gradual fading of the Van Gieson stain is annoying, and may be compensated for by the use of the Ponceau S mixture of Curtis (Carleton, 1938, p. 109). It is said to have the disadvantage of not being as specific for newly formed connective tissue, but it is a permanent stain. Ponceau S is not listed by Conn (1940), and Ponceau B (syn: Biebrich scarlet, water sol.; croceine scarlet; scarlet B, or EC; and double scarlet BSF) has been used in its place.

Summary

A method is described for demonstrating the fibrillar structure of decalcified bone by washing out the amorphous collagen with a 3% KOH solution to which some glycerin is added. When dissociation of the ossein has progressed far enough the specimen is embedded in paraffin, and sections are cut at 10μ to 12μ. The fibrillae are stained with orcein or picro-fuchsin. These stains show a selective specificity that permits a differential staining of the diffuse and compact lamellae in bones having Haversian systems. Bones that do not show such a well-oriented fibrillar structure do not have this differential staining reaction. The use of Ponceau B instead of fuchsin in Van Gieson's stain is suggested to circumvent the tendency of the stain to fade.

BIBLIOGRAPHY

Carleton, H. M. 1938. Histological Technique, *2nd Ed*, Oxford University Press, N. Y.

Conn, H. J. 1940. Biological Stains. Biotech Publications, Geneva, N. Y.

Mall, F. P. 1906. On ossification centers in human embryos less than one hundred days old. Amer. J. Anat., **5**, 433.

Ruth, E. B. 1945. Bone studies, I. Fibrillar structure of adult human bone. Unpublished data.

CARBOL CRYSTAL VIOLET[1]

H. J. Conn, *New York Agricultural Experiment Station, Geneva, N. Y.*

There is some call among bacteriologists for a "carbol gentian violet". It is used sometimes as a general bacterial stain and sometimes in the Gram technic, to be followed by treatment in iodine and decolorization. The use of the formula is generally ascribed to Nicolle (1895), although it is far from certain that he originated the idea. Inasmuch as carbol fuchsin had then been in use for at least a decade, it would be surprising if no one had prepared a similar "gentian violet" formula before 1895. Although a search of the literature would probably bring out some such formula earlier than the paper of Nicolle, none has yet been found, and it has hardly seemed an important enough point to make the necessary search.

The formula first came into use as improvement on Ehrlich's "anilin gentian violet"; and there can be no question but that it is an improvement. It seems to be an equally efficient stain, to work practically the same in the Gram method, and to have the advantage of greater permanency of the solutions. In fact, a properly prepared carbolic crystal violet seems to be about as permanent as a staining fluid can be made; while the anilin-water formula is notoriously unstable. Nevertheless, the writer has never recommended (either personally or on behalf of the Committee on Bacteriological Technic) the use of carbol crystal violet in the Gram stain; the ammonium oxalate formula (Hucker, 1922) has always seemed, and still seems, to be a distinctly more satisfactory staining fluid for the purpose.

The only reason for the present paper on carbol crystal violet is that recent correspondence brings out two facts: first, that in some quarters the formula is in use; second, that difficulties are experienced when making it up either by Nicolle's original formula or by the emended formula recommended by the Committee on Bacteriological Technic. The difficulty frequently observed is that the solutions sometimes tend to gelatinize, often becoming semisolid before they have stood 24 hours.

The original formula of Nicolle called for one volume of saturated alcoholic "gentian violet" to 10 volumes of 1% aqueous phenol. It was found some 20 years ago that satisfactory results were obtained with this formula when approved samples of crystal violet, such as

[1]Journal Paper No. 662, New York State Agricultural Experiment Station, Geneva, N. Y., January —, 1946.

then were on the market, were used for the dye. These samples had a dye content of about 85%. The objection to the formula was that it was indefinite, even when crystal violet was substituted for the inderterminate "gentian violet" originally called for, because a saturated alcoholic solution of crystal violet cannot be regarded as containing a fixed quantity of the dye. Hence an effort was made to state it more definitely by emending the formula to call for a 1% solution in ethyl alcohol of a sample having 85% dye content. Such solutions made up at that time were stable; this proves now to be the case with at least one recently manufactured lot of crystal violet having a dye content of only 88%.

The majority of present-day crystal violets, however, have a dye content of 90 to 95%, and with them the formula in question is less satisfactory. Just why the objectionable gel should form with a 1% solution of one of the present-day more concentrated batches of the dye but not with a saturated solution of the weaker dyes of twenty-odd years ago, is difficult to explain; but it is certainly true that when a 1% solution of one of the batches now on the market, having a dye content of over 90%, is mixed with a 1% aqueous solution of phenol, the fluid gelatinizes when allowed to stand overnight.

The difficulty can simply be avoided by using a crystal violet solution something less than half as strong as that originally recommended. Accordingly the formula now recommended is:

Solution A		Solution B	
Crystal violet CC[2]	0.4g.	Phenol	1g.
Ethyl alcohol (95%)	10. ml.	Distilled water	100ml.

Mix solutions A and B

This formula is recommended merely as an improvement on the Nicolle formula, when carbol crystal violet is desired. It must be distinctly understood, however, that the writer still regards the ammonium oxalate formula as preferable, both in the Gram technic and for ordinary bacterial staining.

REFERENCES

HUCKER, G. J. 1922. Comparison of various methods of Gram staining. (Preliminary report.) Abstr. Bact., **6**, 2.

NICOLLE, CH. 1895. Pratique des colorations microbiennes. Ann. Inst. Past., **9**, 664–70.

[2] *i. e.* Commission Certified.

LABORATORY HINTS
FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

MICROSCOPE AND OTHER APPARATUS

A. B. The Maltwood finder. *Microscope*, **5**, 268. 1945.

The usual type of this finder takes the form of a ruled mesh 50 units square (2500 units). Each unit bears 2 numbers indicating its position relative to the left-hand and upper margins respectively. Thus the third square in the second row is marked $\frac{2}{3}$. The slide (preparation) to be searched is examined with a suitable combination of objective and ocular and moved by means of a mechanical stage or other device, the slide being kept firmly against the stop. When the object whose position is to be recorded is located, the slide is carefully removed and the finder is inserted in its place. The numbered square visible in the field is noted.—*C. E. Allen.*

BROWNELL, CHARLES G. Simplified micro-projection for small groups. *J. Techn. Methods*, **24**, 51–4. 1944.

A monocular microscope and a standard microscope lamp are employed for micro-projection. The microscope is placed in a horizontal position on a table with a lamp behind it, arranged so that the distance from the small hole of the closed lamp diaphragm to the table is equal to that of the closed substage diaphragm to the table. The Kohler method of illumination is recommended. By this method an image of about 24 inches in diameter can be focused on the screen.—*Challiss Randall.*

BURKE, FREDERICK. New form pipette. *Microscope*, **5**, 252. 1945.

One end of a capillary tube (1 mm. bore) is closed and blown out to form a short bulb. The outer half of the bulb is strongly heated and blown off, leaving a short conical end. To this end is sealed a larger bore tube fitting over and extending beyond the opposite end of the smaller tube. A rubber nipple is fitted over the end at which the two tubes are sealed. The device is recommended for handling mosquito and other aquatic larvae.—*C. E. Allen.*

KELEMEN, GEORGE. Microscopy and photomicrography of the temporal bone and polarizing films. *J. Biol. Photog. Assoc.*, **13**, 171–6. 1945.

Polarized light is a great aid in analyzing structural details in general bone histology under normal and pathological conditions. Unfortunately, however, polarizing apparatus is expensive and not always available, but in the greater part of the examinations, it can be replaced by "Polaroid" polarizing films which offer a method of extreme simplicity at negligible expense. A piece of polarized film of the same size as the slide is clamped to the stage of the microscope under the slide carrying the unstained section. This piece serves as a polarizer. A second small round piece is dropped onto the inner diaphragm of the ocular. This serves as an analyzer. By rotating the ocular, opaque and bright positions are produced.

For photomicrography a round piece of the film is fastened in front of the objective and a second piece is slipped into a filter carrier frame between the microscope and the source of light. The second piece is easily rotated. This method is especially desirable because the greatly restricted visual field resulting from the small size of the Nicol prisms is avoided. The author includes photomicrographs to show the use of polarizing film in routine histological analysis of temporal bone.—*Jean E. Conn.*

MICROTECHNIC IN GENERAL

DE ANGELIS, EUGENE. **The use of plastics to replace cover glasses in microscope slide preparations.** *J. Lab. & Clin. Med.*, **30**, 469–71. 1945.

Tissues or other slides ready for mounting are dipped in the plastic for 30 sec., removed, allowed to drain, and dried at room temperature. If the slides are to be filed, they are dried in an oven at 140° C. for one hour. The most satisfactory plastics tried were Acryloid B-72 (Resinous Products and Chemical Co., Philadelphia, Pa.) and Vinylseal (Bakelite Corporation, New York, N. Y.) These adhered well to glass and tissue, had the maximal optical transparency, were resistant to abrasion and common solvents, showed negligible deterioration by light and chemicals, and were easy to apply.—*John T. Myers.*

HALL, WILLIAM E. B. **A method for the preparation of gross demonstration and museum specimens using cross-sectioned tissues.** *J. Techn. Methods*, **24**, 12–20. 1944.

The author describes a method of preparing gross demonstration specimens which consists of placing properly fixed slices of tissue between two sheets of glass, immersing in molten paraffin or paraffin-tissuemat mixture, cooling, and appropriately sealing. Both Nile blue sulphate and scarlet red (Sudan IV) are recommended as fat stains. The technic for Nile blue sulphate is as follows: stain section tissue slices in saturated (less than 2%) aqueous Nile blue sulphate, 15–20 min.; rinse in water; differentiate in 1% aqueous acetic acid, 10–20 min.; wash thoroughly through several changes of distilled water, 1–2 hr.; moisten surface with 4% aqueous formaldehyde. The following technic is advised if Sudan IV is used: moisten briefly in 70% alcohol; immerse in Herxheimer's scarlet red solution, 1–2 min., keeping sections in slight motion; wash briefly in 70% alcohol; wash well in mixture of 1 part 95% alcohol and 1 part 4% formaldehyde until non-fatty tissue becomes white; drain; wash briefly in 4% aqueous formaldehyde. Because of the affinity of this stain for paraffin as well as for fat, speed in the embedding and subsequent hardening is essential and every effort possible should be made to keep the border of the specimen moistened.

Marchi's method, Weigert's method, and LeMasurier's method are all recommended as stains for cerebral and nervous tissue, hematoxylin and Nile blue sulphate for tissues which consist of or include bone.—*Challiss Randall.*

LILLIE, R. D. **Picroformaldehyde formic acid for fixation.** *J. Techn. Methods.*, **24**, 35–6. 1944.

Picro-formalin-formic-acid is a modification of Bouin's fluid which contains 5 parts formic acid, 10 parts strong formaldehyde, and 85 parts saturated aqueous picric acid solution. One change of 70% alcohol is used, then 80%, and the usual acetone gasoline sequence with vacuum infiltration in paraffin. For Romanovsky staining, sections are washed 5–10 min. to remove picric acid. This solution is a good decalcifying agent and avoids over-fixation of the surface layer of solid organs. Otherwise the general effect is similar to that of Bouin's fluid.—*C. Randall.*

DYES AND THEIR BIOLOGICAL USES

ALBERT, A., and GLEDHILL, W. **Improved syntheses of aminoacridines. Part IV. Substituted 5-amino-acridines.** *J. Soc. Chem. Ind.*, **64**, 169–72. 1945.

The reaction whereby 5-chloroacridine is converted into 5-aminoacridine was applied to 24 variously substituted 5-chloroacridines with generally good results, giving the correspondingly substituted 5-aminoacridines, many of which have proved to be powerful antibacterials.—*R. T. Whittenberger.*

MILLIGAN, R. F., and HOPE, F. J. **The structure of dichlorofluorescein.** *J. Amer. Chem. Soc.*, **67**, 1507–8. 1945.

D and C orange No. 8, a new dye permitted by the U. S. Government for use in the manufacture of drugs and cosmetics, was shown to be dichlorofluorescein with the chlorine atoms occupying the 4 and 5 positions in the molecule.—*R. T. Whittenberger.*

WOOD, HARLAND G., and RUSOFF, IRVING I. **The protective action of trypan red against infection by a neurotropic virus.** *J. Exp. Med.*, 82, 297–309. 1945.

Trypan red, when injected intraperitoneally into mice, was found to lower greatly the incidence of infection of mice inoculated intraperitoneally with a virus similar to that of poliomyelitis. The protective action of the dye is overcome if the virus is inoculated in too high concentration. The lowered incidence of infection was observed in mice inoculated with virus as long as 29 days after the last dye injection. Brilliant vital red and Congo red were also found effective. In cotton rats similarly inoculated, trypan red had a similar effect. With monkeys and a typical poliomyelitis virus, no protection was observed, but the experiment was considered inadequate.—*H. J. Conn.*

ANIMAL MICROTECHNIC

HINER, R. L., MADSEN, L. L., and HANKINS, O. G. **Histological characteristics, tenderness, and drip losses of beef in relation to temperature of freezing.** *Food Research*, 10, 312–24. 1945.

Histological sections of meats frozen and examined within 24 to 36 hr. were studied by the following technic:

Frozen blocks of beef were sectioned by a sliding microtome, precooled in a room maintained at approximately −7.8° C., unrolled carefully on a cold slide, thawed by warming the slide from beneath with the operator's fingers, and then placed directly in 70% alcohol, or passed through graded alcohols of 30 and 50% concentration, for about two hours in each, before placing in 70% alcohol. The sections remained in 70% alcohol overnight, then were stained with hematoxylin and eosin, and mounted in the usual manner. Alcohol fixation caused some shrinkage which could be reduced by dipping the sections in 30% alcohol for 3 or 4 sec. to fix the tissue to the slide and then withdrawing and placing in 50% dioxane for 24 hours, after which they were placed in absolute dioxane for another 24 hour period. This latter procedure was followed by the usual staining and mounting technic and was believed to preserve more nearly the original structure of the fiber.

Macroscopic observations of tissues confirmed changes noted in the study of the frozen sections. As freezing temperatures were lowered, precipitated proteins and nuclear fragments outside the fibers became more extensive. Intrafibrillar ice crystals and areas, with fiber-splitting caused by ice formation, were clearly shown.

Frozen meat, thawed, fixed in formalin, dehydrated in dioxane, embedded in paraffin, sectioned and stained, did not preserve the detail observed by the methods described above.—*William G. Walter.*

HOLCOMB, R. C., and APTEKMAN, PAUL M. **A method of preparing fetal bone for exhibition and ready examination by methacrylate.** *J. Techn. Methods*, 24, 21–4. 1944.

The authors describe a technic by which frail fetal bones may be embedded in plastic without distortion. The bone specimens may be stained with alizarin red S and the stain will remain unaltered by the method described. The monomeric ethyl methacrylate used is a clear plastic which transmits every color of the spectrum and which has a light transmission value of 91%. The resin is partially polymerized before the specimen is embedded in it. Polymerization is completed by oven treatment at 55–65° C. for 48 hr. Though some shrinkage occurs, there is no distortion in the specimens.—*Challiss Randall.*

LENDRUM, A. C. **On the "pink" epithelium of the cystic breast and the staining of its granules.** *J. Path. & Bact.*, 57, 267–70. 1945.

Granules in the epithelium of the cystic breast may be demonstrated by the following method: Fix tissue in formol-sublimate; cut paraffin sections as usual. Stain with Mayer's hemalum. Then stain 1–2 min. in 1% aqueous fast green containing 0.5% acetic acid. Rinse in water and immerse in Lugol's iodine 2 min. Rinse in 95% ethanol. Immerse in 2% phosphotungstic acid in 95% ethanol. Rinse briefly in water. Stain in "carbacid fuchsin" 2–6 min., con-

trolling under the microscope. To prepare "carbacid fuchsin", melt 0.4 g. phenol and mix with 1.0 g. acid fuchsin. When cool dissolve in 10 ml. 95% ethanol and make to 100 ml. with starch-dextrin (grind 0.5 g. starch to fine powder, then grind in 0.5 g. dextrin, add gradually with grinding 100 ml. water and heat to 80° C.). When cool filter into the phenol sludge solution. The granules stain blackish-red as a result of taking up some of the fast green together with the subsequent fuchsin.

As the nuclear stain is removed in part by the subsequent treatment with iodine, greater nuclear detail is obtained by staining, prior to the hemalum, with celestin blue (dissolve 2.5 g. iron alum overnight at room temperature in 50 ml. distilled water, add 0.25 g. celestin blue R, C.I. 900, and boil 3 min. Filter, add 7 ml. glycerol).

The green stain is partly removed by iodine. Stronger final green staining of cytoplasm is obtained by increasing the original time of staining. The time in iodine should not be less than 2 min.—*S. H. Hutner.*

LENDRUM, A. C., CARSON, W., and PENNY, W. **On the staining of basement membranes.** *J. Path. & Bact.*, **57**, 270–1. 1945.

The method devised for staining of granules (see previous abstract) was found to give excellent staining of the basal membrane of the renal glomerulus provided the following additional step is performed: Subject paraffin sections before de-waxing to the vapor of hot formalin (heat formalin in a closed dish at 55–65° C. for about 2 hr.). Then stain as already described.—*S. H. Hutner.*

LILLIE, R. D. **Study of certain oil soluble dyes for use as fat stains.** *J. Techn. Methods*, **24**, 37–42. 1944.

Satisfactory fat staining in which lipids are preserved may be obtained in 2 to 5 min. by using a 30:30:40 or 25:25:50 solvent of methyl or ethyl alcohol, acetone and water saturated with Sudan brown (C. I. No. 81), Sudan R (C. I. No. 113), Sudan brown 5B, oil red 4B, oil red EGN, Sudan II (oil red O), (C. I. No. 73) or Sudan black B. Precipitation may be prevented by dipping the sections in the same solvent mixture as used for the dye for 2–3 sec. just before staining. The Sudan browns give a deep brownish red, the oil reds and Sudan R a deep orange red, the Sudan II a light yellowish orange, and the Sudan black B a greenish black. Other good dyes are Sudan III (C. I. No. 248), oil brown M, Sudan Corinth B, and Sudan red 4BA.—*C. Randall.*

LILLIE, R. D. **Various thiazin and fluorane dyes in paired combinations as neutral stains for tissue.** *J. Techn. Methods*, **24**, 43–5. 1944.

A study was made of various commercially available thiazins and eosins as components of the neutral stain in the buffered Romanovsky technic. Both Nocht and Giemsa type technics were used. Sections were brought from paraffin to water in the usual way, stained 1 hr. in the appropriate mixture, rinsed in water, dehydrated with acetone, passed through acetone and xylene into xylene and mounted in clarite. Eosinates were made and 0.2 g. portions dissolved in 100 ml. of a mixture of equal parts of C.P. glycerol and methanol which had been redistilled from silver oxide. Sections were stained at pH 4.6 for 1 hr.

The author recommends the B and Y eosinates of azure A, azure C and toluidine blue.—*C. Randall.*

NEUDA, PAUL M., and ROSEN, MAURICE S. **A preliminary report on a rapid method for diagnosing sickle-cell disease.** *J. Lab. & Clin. Med.*, **30**, 456–8. 1945.

Sickle cell forms appear promptly when a drop of sickle-positive blood is in contact with a broth culture of normal human feces. The procedure used is as follows: Mix a cherry-size piece of feces with 5 ml. of isotonic NaCl solution, stir and filter. Add 0.1 ml. of the filtrate to a tube of nutrient broth and incubate 24 hr. at 37° C. Place a drop of this culture on a slide, add a drop of the suspected blood, apply a cover slip and examine microscopically. There is an agent in the broth which causes susceptible cells to rapidly assume the sickle form.—*John T. Myers.*

ZIEGLER, J. A. **Use of the benzidine staining method for the study of capillaries in the cornea.** *Canad. J. Res., Sec. E,* **23,** 115–17. 1945.

In a study of the metabolism of riboflavin, it was necessary to study the capillaries of rat corneas. As other methods failed to differentiate the vessels from the remainder of the corneal tissue, the following technic was devised: remove the whole eye and fix in 10% formalin-saline fixative, 2 hr.; fix in fresh fixative, not less than 1 week; wash eye; excise cornea; cut cornea in four equally spaced centripetal incisions; flatten out cornea; wash in frequently changed distilled water, 30 min.; stain in freshly prepared Solution A, 20 min.; wash in distilled water, 10 to 15 sec.; place in Solution B, 15 min. at room temp. and then 30 to 45 min. at 37° C.; wash in distilled water, 15 min.; dehydrate in 70 and 95% alcohol, each acidified with 2% acetic acid; dehydrate in neutral 100% alcohol; clear and mount in sandrac, camsal, dioxane mixture. Stained corneas must not be unduly exposed to light. Blood vessels filled with blood are jet black, those not filled are faintly brown, and the background is pale yellow. Solution A is a mixture of 0.5 g. recrystallized benzidine in 50 ml. absolute alcohol and 0.1 g. sodium nitroprusside in 10 ml. distilled water, diluted to 100 ml. Solution B is a mixture of 50 ml. absolute alcohol, 3 ml. glacial acetic acid, 0.5 ml. 30% hydrogen peroxide, and 0.1 g. sodium nitroprusside, diluted to 100 ml. Freshly prepared solutions must always be used, and benzidine must be purified by recrystallization from alcohol.—*H. P. Riley.*

PLANT MICROTECHNIC

ERIKSON, D. **Certain aspects of resistance of plum trees to bacterial invasion of Prunus sp. by Pseudomonas mors-prunorum Wormald.** *Ann. Appl. Biol.,* **32,** 112–17. 1945.

Resistant and susceptible varieties of plums were inoculated with *Pseudomonas mors-prunorum* Wormald and a histological study was made of the necrotic areas induced in the stem. Freehand sections of fresh bark from vicinity of inoculation were fixed in formalin-acetic-alcohol and stained with 1:10,000 aqueous methylene blue. In normal tissues, lignin was green, cellulose blue, and pectic substances violet; in diseased areas, tissues were brown except that lignin of the bundles was green. Paraffin sections made by the *n*-butyl alcohol, cedar oil and paraffin technic were stained with safranin and Harris' hematoxylin or fast green, or with methyl violet and erythrosin. The pathogen penetrates the tissues between the cells, causing plasmolysis of cell contents, disintegration of cell walls, and gradual invasion by bacteria. The details of invasion are the same for both varieties but in the resistant one it progresses to a less degree and periderm formation shuts off the invaded tissue at a very early stage.—*H. P. Riley*

JOHNSON, L. P. V. **A rapid squash technique for stem and root tips.** *Canad. J. Res., Sec. C.,* **23,** 127–30. 1945.

Chromosome studies were made from the growing point of the stem and from root tips by the following technic: fix in Carnoy's fluid, 30 min.; macerate and hydrolyze at room temperature in 1 part concentrated HCl and 1 part 95% ethanol, 5 to 10 min.; rinse in two changes of water as quickly as possible; stain in leuco basic fuchsin, one hour or longer; transfer to drop of 50% acetic acid on slide; cover; squash by tapping with a pencil; apply pressure with finger, protecting cover glass with absorbent paper; allow to stand until drying begins under cover glass, about 10 min.; remove cover glass; flood cover and slide twice with 95% ethanol and three times with *n*-propyl acetate at about half-minute intervals; place drop of Canada-balsam-xylene on slide; replace cover glass. The leuco basic fuchsin is prepared as follows: dissolve 1 g. basic fuchsin by pouring 200 ml. boiling distilled water over it; shake thoroughly; cool to 50° C.; filter; add to filtrate 2 g. potassium metabisulphite and 20 ml. *N* HCl; shake; stopper; bleach in the dark, 24 hr.; add 0.5 g. decolorizing carbon; shake one minute; filter through coarse filter paper. The new feature of this method is the use of *n*-propyl acetate as a dehydrating agent because it is far less toxic than is 1,4-dioxane. It was especially good for species of *Abies, Picea, Pinus, Pseudotsuga, Thuja,* and *Tsuga.* For tender material, as tomato root tips, it was preferable to reduce the strength of the macerating agent to equal proportions of 95% ethanol and 25% HCl.—*H. P. Riley.*

MICROÖRGANISMS

CROSS, JOY BARNES. **A rapid fixation technique for Trematoda and Hirudinea.** *Texas Rept. Biol. and Med.*, **3**, 101–4. 1945.

A method is described by which ninety well flattened liver flukes may be prepared in an hour, which is considerably faster than the usual procedure of tying the animals between glass slides. Rapidity is particularly important because the period of survival after the host's death is short and only live specimens can be used.

The procedure used is as follows: Tilt a dark-bottomed dissecting pan containing a small amount of SUSA fixative in such a way that the upper two-thirds of the pan remains dry. Place a strip of toilet paper in the dry portion, lay a fluke on one end, and brush flat with a camel's hair brush. Fold the opposite end of the paper over the animal, brush a few times, and cover with a piece of glass leaving some of the paper exposed. While pressing the brush handle against the glass with one hand, use the other to grab the exposed paper with paraffin-tipped forceps and to pull the preparation downward into the fixing solution. The weight of the glass is sufficient to maintain the flattened state while other flukes are prepared in the same way. When the first one has been in the solution long enough to become opaque and to retain its flattened condition, remove the glass and surrounding paper and transfer to a larger container of fixing solution for approximately 12 hours.

With this method, fixation is achieved superficially in 2 or 3 minutes whereas the older method requires 4 hours. The shorter time is especially desirable because it is generally recognized that rapid fixation minimizes the production of artifacts. This method can also be used for leeches and probably for other Trematodes.—*Jean E. Conn.*

SAMUEL, G., and GARRETT, S. D. **The infected root-hair count for estimating the activity of Plasmodiophora brassicae Woron. in the soil.** *Ann. Appl. Biol.*, **32**, 96–101. 1945.

To study the effect of soil conditions upon the incidence of a soil-borne parasite, it is necessary to measure disease intensity in the soil. The intensity of infection of cruciferous seedlings by *Plasmodiophora brassicae* Woron. can be measured by counting the number of infected root hairs on the tap roots of young seedlings by the following technic: inoculate tumblers of soil with a spore suspension; plant 12 presoaked cabbage seeds in each tumbler; cover with 0.25 cm. soil; cover tumblers with glass lids and keep at 25° C. for 1 week; wash out; drop the 5 strongest seedlings into 1% aceto-carmine where roots may be left for several months; place tap roots under long cover glass on microscope slide ruled at 1 mm. intervals; count under 1 in. objective and 10× ocular using mechanical stage the number of root hairs containing zoosporangia along 2 cm. of seedling tap root; infected root hairs are counted on each side of the root only and not on branch rootlets. This method makes it possible to study initial stage of infection with greater quantitative accuracy than by any previous method.—*H. P. Riley.*

SEMMENS, C. S. **Pythium de Baryanum.** *Microscope*, **5**, 257–64. 1945.

The relation of the fungus to the host can be observed in a portion of the root of an infected cress seedling. The best part to use for this purpose extends for about a centimeter above the point of attachment to the remains of the seed. This is placed on a slide, covered with a drop of cotton blue in lactophenol; after a few minutes the dye solution is replaced with plain lacto-phenol. The hyphae of the fungus are stained deep blue; the host tissues are unstained or only faintly tinged.—*C. E. Allen.*

HISTOCHEMISTRY

HAUSER, E. A., and LE BEAU, D. S. **Microscopic studies of lyogels. Ultra-illumination by incident light.** *Ind. Eng. Chem.*, **37**, 786–9. 1945.

The preparations are made by dispersing or dissolving the colloid to be studied in an appropriate solvent and spreading this solution on a nonmiscible liquid. A piece of fine wire gauze is then coated by being lifted through the solution, and is placed on a microscope slide. The Ultropak ultramicroscope, which gives

circular illumination of the preparation from above, is used. Diffusion of light caused by reflections from uneven surfaces or from opaque specimens can be avoided, and various micromanipulations under the microscope are possible without blurring of the image.—*R. T. Whittenberger.*

JOYET-LAVERGNE, P. **La mise en evidence d'une action de la vitamine C sur la cellule vivante, grace a l'intervention de l'adrenaline.** *Compt. Rend. Acad. Sci.*, **217**, 327. 1943.

A thin section of living tissue is divided into three parts. One part is placed in water, the other two in a drop of adrenaline in a 1 to 1000 dilution. After 8 minutes a drop of water is added to the sections placed in water and to one of the sections placed in adrenaline; a drop of ascorbic acid (1 to 1500) is added to the second adrenaline preparation. After 10 min. a quantity of a leucoderivate of one of the following dyes is added to each of the three preparations: phenosafranin, neutral red, cresyl blue and Nile blue. While the type of changes occurring in the various preparations are not described, the author states that they indicate that adrenaline slightly increases the intracellular oxidation and that adrenaline plus ascorbic acid greatly increases it.—*Elliott Weier.*

SERRA, J. A. **Improvements in the histochemical arginine reaction and the interpretation of this reaction.** *Portugaliae Acta Biol.*, **1**, 1–7. 1944.

The author reviews the directions for the histochemical detection of arginine, previously published by himself and Queiroz Lopes, as follows: Fix tissue in alcohol-formalin; or if fixed in some other cytological fixative, harden the fixed tissue 12–24 hr. in 1:3 formalin. Immerse pieces in a mixture of the following: 0.5 ml. of 1% α-naphthol in 96% alcohol, diluted 1:10 in 40% alcohol just before use; 0.5 ml. *N* NaOH; 40% aqueous urea. After 12–15 min. add 2% sodium hypobromite (prepared by throwing 2 g. liquid bromine into 100 ml. of 5% aqueous NaOH, agitating and cooling), and allow to act for 3 min. Then add 0.2 ml. of the urea solution and 0.2 ml. of the hypobromite solution with constant stirring; after 3–5 min. the color has reached its maximum. Then remove pieces and place for 2–3 min. in pure glycerol, then changed to fresh glycerol. Make cytological preparations by squeezing or smearing as for aceto-carmine. Or, if desired, make permanent preparations by passing the smears or very thin pieces through 96% alcohol into some alcoholic mounting medium such as Euparal.

The resulting red-orange coloration is characteristic of arginine and is given by only a few other rare compounds (glicociamine, galegine, agmatine) that can occur in biological materials. In practice therefore, it is a specific histochemical reaction for protein-arginine. Several photomicrographs are given showing the presence of arginine, demonstrated by this technic, in certain cytological material.—*H. J. Conn.*

SERRA, J. A. **Sur la composition protéique de chromosomes et la réaction nucléale de Feulgen.** *Bol. Soc. Broteriana* (Portugal), **17**, 203–16. 1943.

Using histochemical methods, the writer makes an investigation of the nature of chromosomes and of the significance of the Feulgen reaction. He shows that the proteins of the metaphase chromosomes are chiefly of the basic type, having a high percentage of arginine. Tests to show the existence of an acidic protein, chromosomine (claimed by the Stedmans to constitute more than 50% of the chromosome substance) have not been conclusive. At present it is not possible to assert that chromosomine or any other acidic protein is an important constituent of the metaphase chromosomes. The nucleal reaction of Feulgen is perfectly localized, and is a positive reaction to demonstrate the presence of thymonucleic acid, *in situ*. The staining with fuchsin after treatment with nucleases proves that the nucleal reaction is not due to an assumption of color by an acidic protein. —*H. J. Conn.*